

What is claimed is:

1. A method for designing primer pairs for amplifying a target sequence, comprising the steps of:

choosing a reference sequence;

removing at least selected repeat regions in said reference sequence to yield removed and unremoved reference sequence;

selecting primer sequences from said unremoved reference sequence according to two or more parameters including primer length and primer melting temperature to yield a set of primers;

evaluating said set of primers for extent of coverage and overlap of said reference sequence; and

selecting a subset of primer pairs having reduced overlap from said set of primers.

2. The method of claim 1, wherein said primer length is selected to be between about 28 nucleotides and about 36 nucleotides.

3. The method of claim 1, wherein said primer melting temperature is between about 72 °C and about 88 °C.

4. The method of claim 1, wherein said two or more parameters from said first selecting step is selected from the group of stringency, duplex existence, specificity, GC clamp, hairpin existence, sequence repeat existence, dissociation minimum for 3' dimer, dissociation minimum 3' terminal stability range, dissociation minimum for minimum acceptable loop, percent maximum homology, percent consensus homology, maximum number of acceptable sequence repeats, frequency threshold, and maximum length of acceptable dimers.

5. The method of claim 1, wherein said extent of coverage is above about 90% of said reference sequence.

6. The method of claim 1, wherein said extent of overlap is less than about 5% of said reference sequence.

7. The method of claim 1, wherein said removing step is performed by a computer program.

8. The method of claim 7, wherein said computer program is RepeatMasker.

9. The method of claim 1, wherein said first selecting step is performed by a computer system.
10. The method of claim 9, wherein said computer system is selected from the group of Oligo, xprimer, PrimerSelect, and Primer 3.
11. The method of claim 1, wherein said second selecting step selects a subset of primer pairs with a minimal or substantially minimal number of primer pairs required to amplify said target sequence.
12. The method of claim 11, wherein said second selecting step selects a subset of primer pairs with a least number of primer pairs required to amplify said target sequence.
13. The method of claim 11, wherein said second selecting step selects said subset of primer pairs according to at least one parameter selected from the group of overlap length, gaps between pairs of primer pairs, and necessity of adding another primer pair to the subset.
14. The method of claim 1, 11, 12 or 13, wherein said second selecting step is performed by a computer system.
15. The method of claim 11, wherein said computer program executes a single-source shortest-path algorithm to select said subset of primer pairs.
16. The method of claim 15, wherein said computer program executes an algorithm solving a single-source shortest path problem on a weighted, directed graph  $G=(V,E)$  for the case in which all edge weights are nonnegative, and  $w(u,w) \geq 0$  for each edge  $(u,v) \in E$ .
17. The method of claim 11, wherein said computer program executes a greedy algorithm to select said subset of primer pairs.
18. The method of claim 1, wherein said target sequence is genomic DNA from a human species.
19. The method of claim 1, wherein said target sequence is genomic DNA from a non-human primate species.
20. The method of claim 1, wherein said reference sequence is genomic DNA from a human species.
21. The method of claim 1, wherein said primer length is about 28 nucleotides to about 36 nucleotides and said melting temperature is about 72 °C to about 88 °C.

22. A computer program for designing primer pairs for amplifying a target nucleic acid sequence comprising:

computer code that receives input of a reference sequence;

computer code that removes at least selected repeat regions in said reference sequence to yield removed and unremoved reference sequence;

computer code that selects primer sequences from said unremoved reference sequence according to two or more parameters including primer length and primer melting temperature to yield a set of primers;

computer code that evaluates said set of primers for extent of coverage and overlap of said reference sequence; and

computer code that selects a subset of primer pairs having reduced overlap from said set of primers.

23. The computer program of claim 22, wherein said computer code for said removing step references a database.

24. The computer program of claim 23, wherein said database is RepBase.

25. The computer program of claim 24, wherein said computer program comprises RepeatMasker.

26. The computer program of claim 22, wherein said computer code that selects primer sequences in said first selecting step uses additional parameters selected from the group of stringency, duplex existence, specificity, GC clamp, hairpin existence, sequence repeat existence, dissociation minimum for 3' dimer, dissociation minimum 3' terminal stability range, dissociation minimum for minimum acceptable loop, percent maximum homology, percent consensus homology, maximum number of acceptable sequence repeats, frequency threshold, and maximum length of acceptable dimers.

27. The computer program of claim 22, wherein said computer code comprises code selected from the group of Oligo, PrimerSelect or Primer 3.

28. The computer program of claim 22, wherein said computer code executes an algorithm that in said second selecting step selects a subset of primer pairs with a minimal or substantially minimal number of primer pairs required to amplify said target sequence.

29. The computer program of claim 22, wherein said computer code executes an algorithm that in second selecting step selects said subset of primer pairs according to at least one parameter selected from the group of overlap length, gaps between pairs of primer pairs, and necessity of adding another primer pair to the subset.
30. The computer program of claim 22, wherein said computer code executes a single-source shortest-path algorithm.
31. The computer program of claim 22, wherein said computer code executes Dijkstra's algorithm.
32. A system that designs primer pairs for amplifying a target nucleic acid sequence comprising:  
a processor; and  
a computer readable medium coupled to said processor for storing a computer program comprising: computer code that receives input of a reference sequence; computer code that removes at least selected repeat regions in said reference sequence to yield removed and unremoved reference sequence; computer code that selects primer sequences from said unremoved reference sequence according to two or more parameters including primer length and primer melting temperature to yield a set of primers; computer code that evaluates said set of primers for extent of coverage and overlap of said reference sequence; and computer code that selects a subset of primer pairs having reduced overlap from said set of primers.